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TITLE: Prostate-Specific Membrane Antigen (PSMA) Targeted Bio-orthogonal Therapy for Metastatic Prostate Cancer

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14. ABSTRACT

During the first year of the project we have identified new monoclonal anti-PSMA antibody (mAb), which demonstrated high affinity to the PSMA receptor and excellent targeting of PSMA-expressing prostate cancer cells both *in vitro* and *in vivo*. We investigated details of the mAb and therapeutic complexes internalization in these cells and demonstrated rapid perinuclear localization of internalized agents. We have also synthesized and tested click-reactive components for *in vivo* therapy. First *in vivo* data for anti-PSMA click-based pretargeting have been obtained with NIR *in vivo* optical imaging. In addition, all regulatory reviews of the proposed animal procedures have been successfully completed.

15. SUBJECT TERMS

Targeted agents, anti-PSMA antibody, image-guided therapy

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1. Introduction

During the first year of the project we have identified new monoclonal anti-PSMA antibody (mAb), which demonstrated high affinity to the PSMA receptor and excellent targeting of PSMA-expressing prostate cancer cells both *in vitro* and *in vivo*. We investigated details of the mAb and therapeutic complexes internalization in these cells and demonstrated rapid perinuclear localization of internalized agents. We have also synthesized and tested click-reactive components for *in vivo* therapy. First *in vivo* data for anti-PSMA click-based pretargeting have been obtained with NIR *in vivo* optical imaging. To further understand the tumor delivery patterns contrast enhanced MRI was performed in several animals with PC3-Luc and PC3-PIP tumors. In addition, all regulatory reviews of the proposed animal procedures have been successfully completed.

2. Keywords

3.

Anti-PSMA monoclonal antibody, targeted therapy, *in vivo* imaging, antibody internalization, cytotoxic conjugates, two-component therapy, pretargeting

4. Accomplishments

What were the major goals of the project?

Specific Aims of the proposal are:

Aim 1. Synthesize and characterize anti-PSMA J591 mAb-based pretargeting and albumin based nanocarrier components. Optimize the therapeutic efficacy of the delivery strategy *in vitro* in PSMA(+) and PSMA(-) PCa cells.

Aim 2. To evaluate the therapeutic system in subcutaneous and intratibial metastatic mouse models using PSMA(+) C4-2 and PC3-PIP and PSMA(-) PC3 cell lines.

Specifically for the first year of the project the approved SOW includes the following activities:

Subtask 1: Synthesize TCO-functionalized anti-PSMA antibody and tetrazine-functionalized HSA-DM1 carriers.
Subtask 2: Characterize stability, solubility, and affinity of specific binding of components in a panel of PSMA-positive cancer cells.
Subtask 3: Study mechanism of internalization of the therapeutic complexes in PSMA-positive cancer cells.
Subtask 4: Regulatory review of the animal procedures proposed.

What was accomplished under these goals?

TCO-fucntionalized anti-PSMA antibodies were synthetized using parent 5D3 mAbs, functionalized TCO moieties for click chemistry reactivity, and fluorophores for microscopy (AF488) and *in vivo* NIR fluorescence imaging (CF680). Examples of *in vitro* and *in vivo* binding of the functionalized antibody in PSMA-expressing (high and low) prostate cancer cells and experimental tumors are shown in Fig. 1.

To synthesize cytotoxic drug carrier components globular albumin molecules were conjugated with PEGylated tetrazine (Tt, reactive group for click chemistry) and microtubule inhibitor mertansine (DM1) therapeutic agent using MCC hetero-bifunctional linker. The high-molecular weight conjugate was purified by ultrafiltration and size-exclusion chromatography.

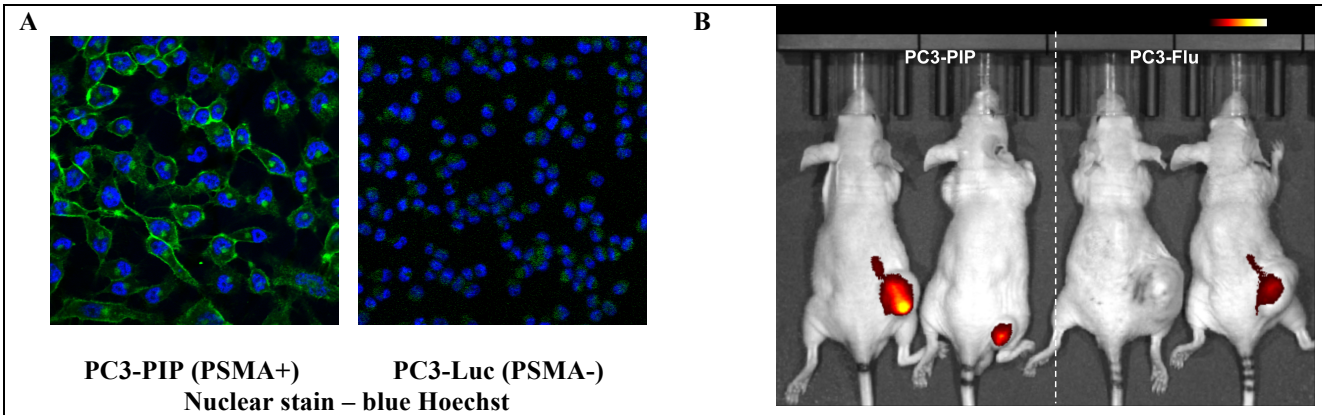

Figure 1. Specific uptake of fluorescent 5D3-TCO mAb in PC3- PIP (PSMA+) and PC3-Flu (PSMA-) cells (A) and experimental tumors (B).

Mass-spectroscopy of parent albumin, intermediate compounds, and final product are shown in Fig. 2. All compounds were stable for at least 24h in sterile buffers and media at room temperature and at least for 7 days at 4^0C. Internalization of components was studied in details in PSMA+ PC3-PIP cells and we demonstrated perinuclear localization of the internalized complexes presumably in the centrosome of the target cell (Fig. 3 - green). The internalization mechanism is different from clathrin-mediated endocytosis, as one can see by comparing signals of internalized dextran and mAbs (Fig. 3 - red).

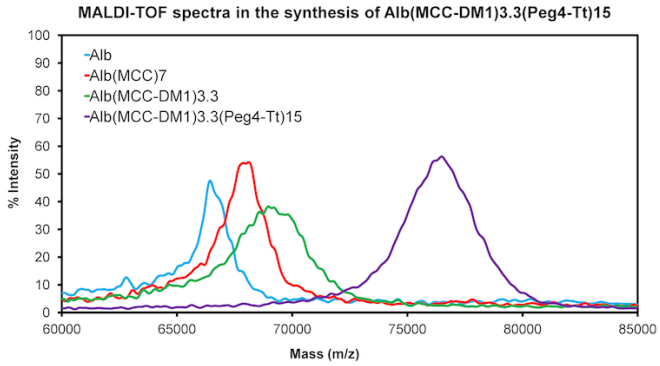

Figure 2. MALDI-TOF mass spectra of the parent albumin, intermediates, and final cytotoxic reactive conjugate.

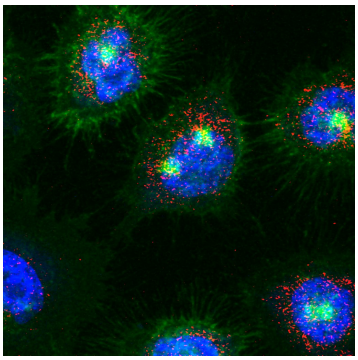

Figure 3. Confocal microscopy of internalization of therapeutic components by PC3-PIP prostate cancer cells. Green – internalized mAb, red-dextran molecules, blue – cell nuclei.

This new mechanism opens an interesting possibility to deliver therapeutic compounds to the perinuclear location without exposing them (or the carrier) to degradative proteolytic enzymes present in endosomes and lysosomes.

Our most recent NIR imaging data for *in vivo* distribution of pretargeting and drug carrier components are shown in Fig .4.

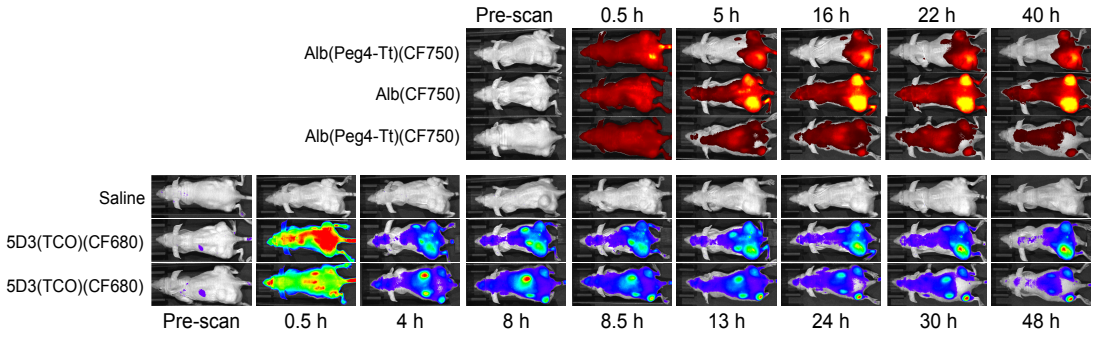

Figure 4. Uptake of functionalized 5D3 mAb (lower raw) and the drug-carrier albumin component (top raw) in representative mice with both PC3-PIP and PC3-Flu xenografts.

Deliverables: during the first year of the project we have synthesized and characterized in vivo and in vitro the following therapeutic components:

(i) 5D3(mAb)-TCO-CF680 for *in vivo* NIR fluorescent imaging
(ii) 5D3(mAb)-TCO-Alexa488 for confocal microscopy
(iii) Albumin-PEG4-Tt-CF750 for *in vivo* NIR fluorescence imaging
(iv) Albumin-PEG4-Tt-Rhodamine for confocal microscopy
(v) Albumin-PEG4-Tt-MCC-DM1 cytotoxic drug carrier component

How were the results disseminated to communities of interest?
Scientific presentations were given at laboratory seminars and some results presented at JHU ICMIC seminar series

What do you plan to do during the next reporting period to accomplish the goals?
During the next period of the project (year 2) we will test the synthesized cytotoxic conjugates in cultured prostate cancer cells with different levels of expression of PSMA-receptors. We will optimize the compounds for *in vivo* studies in orthotopic and metastatic mouse models of human prostate cancer. Intratibial models of metastatic prostate cancer will also be developed.

5. Impact

What was the impact on the development of the principal discipline(s) of the project?
Novel two-component pretargeting system for specific therapy of PSMA-expressing prostate cancer can result in high efficacy and significantly reduced toxicity and side effects. Our synthesized therapeutic components (pretargeting and drug-carrier) are a first generation of such a system and are applicable to experimental therapy in animal models. Once optimized and validated future translation to clinic should be feasible.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

6. Changes/Problems

In the course of the study we became aware of a novel monoclonal anti-PSMA antibody, 5D3, produced by Dr. Barinka (Institute of Biotechnology CAS, Vestec, Czech Republic), which have been used in the lab of our consultant, Dr. M. Pomper. These antibodies are significantly less expensive then originally proposed J591 mAb, and have been extensively characterized both *in vivo* and *in vitro* and have similar or better affinity to the extracellular domain PSMA target than other anti-PSMA antibodies. The 5D3 antibody is also available in large batches ($\geq$ 10mg). Therefore we have used and planning to use this mAb in all our *in vitro* and *in vivo* studies for therapeutic PSMA targeting

7. Products

Research materials (please see Accomplishments)

8. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Dmitri Artemov, Ph.D.	Sean Lupold, Ph.D.	Sudath Hapuarachchige, Ph.D.	Colin Huang, B.S.	Martin Pomper, M.D./Ph.D.
Project Role:	PI	Co-investigator	Research Associate	Technician	Consultant
Research Identifier:					
Nearest person month worked:	3	0.6	6	6	
Contribution to the project:	*In vivo* imaging, data interpretation, supervising personal and insuring stable workflow	Providing prostate cancer cells, interpreting *in vitro* results	Chemical synthesis and characterization of therapeutic components, *in vivo* imaging	Maintaining cell cultures, performing routine tests, confocal microscopy and image processing	Consultancy in PSMA targeting and imaging
Funding support:	NIH/NCI	NIH/NCI	NIH/NCI	NIH/NCI	NIH/NCI

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Nothing to report

9. Special Reporting Requirements

Nothing to report

10. Appendices

N/A